REMARKS

Claims 2-13, 16, and 19-23 are currently pending in the application. In order to advance prosecution, Applicant has amended claim 20 and 21. A complete listing of all the claims, in compliance with the revised amendment format, is shown above.

The amendments to the pending claims are made without prejudice, do not constitute amendments to overcome any prior art rejections under 35 U.S.C. § 102, and are fully supported by the specification as filed. For example, support for the phrase "identifying the pixels corresponding to the cellular area to be determined" appears at, *inter alia*, page 7, line 23 to page 8, line 6. Further support for all of the claim amendments can be found throughout the specification.

In the event that Applicants amendments and arguments do not overcome the asserted grounds of rejection, or if they raise additional issues regarding the patentability of the pending claims, Applicants request the opportunity for an interview with the Examiner, preferably in person, to expedite consideration and allowance of their claims.

Discussion of the 35 U.S.C. § 103(a) Rejections

Claims 2-11, 13, 16, and 19-23 are rejected under 35 U.S.C. § 103(a) as being obvious over Slamon *et al* (U.S. Patent No. 5,846,749) ("Slamon") in view of Veltri et al. (U.S. Patent No. 6,463,438) (Veltri). Applicant respectfully traverses this ground of rejection .

Applicants present herewith amendments to independent claim 21. These amendments are made in order to clarify the metes and bounds of Applicants' claims, and as a result of a careful consideration of the points and objections raised in the Office Action. Applicants this make these amendments to ensure that their claims particularly point out and distinctly claim

their invention, and to undo any confusion occasioned by the claims as pending before their amendment herein.

The instantly claimed invention is directed towards methods for determining the quantity of a target protein in cells of a biological sample. This method requires, among other things, that the pixels corresponding to the cellular area to de determined be identified and that the average optical density of stained target protein per pixel of cellular area be determined by, for example, image analysis. Because it is the average optical density of stained protein per pixel of cellular area that is detected, the actual individual cells need never be specifically identified and the number of cells present in the image field need never be actually determined. This determination is followed by either generating a calibration curve relating the known quantity of said target protein with the average optical density of stained target protein per pixel of cellular area, or using such a calibration curve to determine the quantity of a target protein in a biological sample.

An analysis for obviousness requires a determination of the scope and content of the prior art, the differences between the prior art and the claims at issue must be ascertained, and the level of ordinary skill in the pertinent art must be resolved. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). To establish a *prima facie* case of obviousness, the Office must show three basic criteria: (1) there must be a suggestion or motivation to combine the reference teachings; (2) there must be a reasonable expectation of success; and (3) all of the claimed limitations must be taught or suggested in the combined prior art references. M.P.E.P. § 2143.

None of the cited references, alone or in combination, teach or suggest the instantly claimed method. As acknowledged by the Office Action, Slamon does not teach a method of determining an "average optical density of stained target protein per pixel of cellular area." Instead, Slamon quantitates surface membrane and cytosolic proteins by determining the signal

value from a *known* number of cells, and relates this value to values obtained with control cells. Thus, according to the Slamon reference the signal value *per cell* is determined, rather than the average optical density of stained target protein *per pixel* of cellular area. In fact, the Slamon reference does not teach how to quantitate cellular proteins using image analysis *without* knowing or determining the number of cells that are immunostained. Moreover, the Slamon reference does not teach how to determine the "average optical density of stained target protein per pixel of cellular area," let alone how to use that information to determine the quantity of a target protein in cells of a biological sample. Consequently, Slamon does not teach every limitation of the present invention.

The Office Action asserts that because the presently claimed invention is written in open language, the claimed invention does not exclude, *inter alia*, determining optical values from a known number of cells. However, while the Office Action correctly points out that, as written, the pending claims do not exclude the possibility that the optical values from a known number of cells can be determined, such a measurement is completely irrelevant for the present invention. Instead, what is required is that the average optical density of stained target protein per pixel of cellular area be determined, which is not taught by Slamon, as the Office has already acknowledged.

Further, the Office Action argues that merely pointing out that Slamon does not teach a method of determining an "average optical density of stained target protein per pixel of cellular area," amounts to arguing against the reference individually. However, the analysis of Slamon was made in order to determine the scope and content of this prior art reference, a requirement for an obviousness determination. *See Graham,* 383 U.S. at 17. Applicants address below the failure of the Veltri reference to overcome the deficiencies of the Slamon reference if the

teachings of these references were to be combined. Nevertheless, the Office has explicitly acknowledged the deficiencies of Slamon ("Slamon et al. differ from the instant invention in failing to disclose determining optical density of stained target protein per pixel of cellular area in plurality of control cell pellets," Office Action mailed December 16, 2003, p. 6), and these observations are not overcome by combination with the Veltri reference.

The deficiencies of Slamon are not overcome by combination with the Veltri reference because the Veltri reference merely describes a neural network used in a system to detect abnormalities in cells. *See Veltri*, Abstract. The Veltri disclosure relates "to a neural network-based image recognition system for cancerous tissue cell detection." *Id.* at column 1, lines 7-10. In fact, Veltri is described as an advance in automating detection of individual cells, because the visual "inspection of thousands of cells" required in conventional staining techniques is labor intensive, and "the tedium and fatigue imposed upon the technician and the cytopathologist result in a high false negative rate" for the conventional techniques. *Id.* at column 1, lines 30-58.

The Office Action acknowledged that Veltri was only cited for allegedly teaching "determining optical density of stained target protein per pixel of cell area, i.e. nuclear or cytoplasmic receptor sites, in a population of nucleated cells after immunostaining the target biomarker protein in the cells using biomarker specific antibody." *See* Office Action mailed September 10, 2004, at 5. The Office has asserted that support for this proposition can be found at column 5, lines 1-5, and column 9, line 42 to column 10, line 62. *See* Office Action mailed December 16, 2003, at 6. Applicant respectfully asserts, however, that they have ben unable to find support for this assertion in these specific citations, much less anywhere in the entire Veltri reference;. As already noted, Veltri discloses a method for the detection of abnormalities in individual cells. Specifically, Veltri provides a neural network "which performs recognition of

cancerous cells using information derived from an image of the cells, among others, the area, the average intensity, the shape, the texture, and the DNA content (pgDNA) of the cells." *Id.* at column 3, line 66 to column 4, line 4. The cited section of Veltri, Column 9, line 42 to column 10, line 62, merely describes how to obtain two types of information about the individual cells being analyzed: texture and pgDNA. As described in Veltri, "[r]egular texture has more or less periodical patterns, while random texture is best described by its 'coarseness.'" *Id.* at column 8, lines 50-52. Therefore, to measure texture, Veltri describes applying a convolution mask across every pixel in the interior of a cell image, in order to determine the interdependent characteristics of pixels within a neighboring area. *Id.* at column 8, lines 49-66. In other words, the grey-levels (optical densities) of the pixels of the cell image are compared to the gray-levels of neighboring pixels. *See id.*, column 9, line 42 to column 10, line 16. In fact, instead of teaching immunohistochemical staining for such measurement, as required by the present claims, the example cited by the Office Action actually describes using the Feulgen staining procedure, which "specifically stains DNA contents based upon a hydrolysis of nucleic acid and subsequent Schiff-based reaction with free aldehyde groups on the nucleic acid," see column 4, line 60-67. Thus, the Veltri reference in fact teaches detection of DNA staining, not protein staining as required by the pending claims, and therefore this section of Veltri does not teach, much less suggest or motivate, determining an average optical density of stained target protein per pixel of cellular area.

The Office Action's citation of the determination of pgDNA, or the DNA content of individual cells, is equally inapplicable to the present invention. pgDNA relates pixel measurements within an individual cell to "cell constituents such as total amount of DNA in nucleated cells, the amount of monoclonal antibody attached to nuclear or cytoplasmic receptor

sites, etc." *See id.* at column 10, lines 17-21. In other words, instead of teaching the average optical density of stained target protein *per pixel* of cellular area, as required by the claimed invention, Veltri teaches "the summation of the pixel[]" measurements within an individual cell to determine amount of cellular constituents *per cell*. *See id.*, column 10, line 58-62. Moreover, despite the Office Action's characterization of this measurement as disclosing or teaching the determination of the optical density of stained target protein per pixel of cell area, i.e. nuclear or cytoplasmic receptor cites, Velti does not actual teach how to identify pixels corresponding to the cellular area to be determined, much less suggest that it is necessary to only look at the optical density of such pixels. Thus, this section of Veltri also does not teach, much less suggest, determining an average optical density of stained target protein per pixel of cellular area.

Based on the deficiencies of both of the cited references, taken alone or in combination, Applicants respectfully contend that the Office Action has failed to establish a *prima facie* case of obviousness since there is no teaching, suggestion or motivation to combine the cited references, nor would the combination of the reference produce the claimed invention even if made. The Office Action simply asserts without citation to specific evidence of record that it would have been obvious to incorporate the teaching of Veltri in determining optical density of stained target protein per pixel of cell areas "into the method of Slamon because Veltri specifically taught that the resulting values of such pixel measurement related specifically to the target proteins specific in selected cell areas," and therefore "excludes measurement of nonspecific extraneous proteins. *See Office Action*, at 7. Even assuming Veltri did, in fact, teach determining optical density of stained target protein per pixel of cell areas, the reference clearly did not teach that the resulting values of such pixel measurements relate specifically to target

proteins specific in selected cellular areas. Further, Veltri does not teach, much less suggest, that any measurements would exclude nonspecific extraneous proteins, especially the measurements presently claimed. Without providing any support or citation for such an assertion, the Office Action has failed to establish *prima facie* obviousness of the pending claims in view of the cited references. Pursuant to the provisions of 37 C.F.R. §1.104(d)(2), Applicants request that the Examiner disclose any basis known to her supporting said combination of references.

For the reasons set forth above, neither Slamon nor Veltri, cited in support of this ground of rejection, taken either alone or in combination, disclose, suggest or motivate the skilled worker, either individually or in combination, to a method for determining the quantity of target protein in cells of a biological sample by, *inter alia*, identifying pixels corresponding to the cellular area to be determined be identified and determining the average optical density of stained target protein per pixel of cellular area.

Applicants respectfully mreiterate their previously-submitted contention that the arguments supporting the obviousness determination contained in the Office Action are the product of impermissible hindsight. In response to Applicants' previous hindsight contention, the Action asserted that hindsight is appropriate in this case, citing *In re McLaughlin*, 170 U.S.P.Q. 209 (C.C.P.A. 1971). However, in startling contrast to the instant situation, the subject matter at issue in *McLaughlin* was an improved construction arrangement for railroad "boxcars." *Id.* at 210. Even the Court acknowledged that the application at issue in that case involved "only relatively simple mechanical concepts." On the other hand, the present invention relates to a much more complex technology, involving aspects of cell biology, optics, immunohistochemistry and immunological staining, and biological variability. If these distinctions were not enough, the Federal Circuit has made clear that '[o]ne cannot use hindsight reconstruction to pick and choose

among isolated disclosures in the prior art to deprecate the claimed invention.'" *In re Fritch*, 23 U.S.P.Q.2d 1780, 1784 (Fed Cir. 1992) (citations omitted) (quoting *In re Fine*, 5 U.S.P.Q.2d 1596, 1600 (Fed. Cir. 1988)).

Applicants respectfully submit that what the law precludes is precisely the basis for the asserted obviousness rejection. The Action proffers one reference, Slamon, that is admittedly deficient in teachings specifically lacking in the prior art reference, and supplements this teaching with the Veltri reference, with no evidence of record that one of ordinary skill would combine these references. Moreover, even if properly combined the references would not make the instantly-pending claims obvious, since the deficiencies of the Slamon reference are not overcome by combination with the teachings of the Veltr reference, all as outlines in detail above. Accordingly, Applicants respectfully request withdrawal of this rejection and requests reconsideration of the claims.

Claim 12 is rejected under 35 U.S.C. 103(a) as being unpatentable over Slamon in view of Veltri as applied to claims 2-11, 13, 16, and 20-23, and further in view of McNamara et al. (U.S. Patent 6,007,996) ("McNamara"). Applicants respectfully traverse this ground of rejection.

The instantly claimed invention is directed to a method for determining the quantity of a target protein in cells of a biological sample. As stated above, this method requires, among other things, that the pixels corresponding to the cellular area to be determined be identified and that the average optical density of stained target protein per pixel of cellular area be determined by, for example, image analysis. In addition, as the Office Action notes, claim 12 requires, among other things, that the biological sample be stained with a multiplicity of stains.

None of the cited references, either alone or in combination, teach the claimed invention. The teachings and deficiencies, as related to the present invention, of Slamon and Veltri are discussed above, and apply with equal force to this ground of rejection. The Office Action acknowledges that Slamon and Veltri differ from the invention recited in claim 12 because the references fail to disclose staining the biological sample upon which image analysis is performed with multiplicity of stains. The deficiencies of Slamon and Veltri are not overcome by combination with McNamara. McNamara discloses, among other things, a method of *in situ* analysis of a biological sample comprising the steps of staining the biological sample with at least three stains, and collecting spectral data from the stained biological sample, where the spectral data device can collect data from all the stains. *See McNamara*, column 55, line 66-column 56, line 24. McNamara does not provide any teaching whatsoever related to identifying pixels corresponding to the cellular area to be determined be identified or to determining the average optical density of stained target protein per pixel of cellular area. Therefore McNamara does not render claim 12 obvious when combined with the Slamon and Veltri references, and none of the cited art provides the required teaching, suggestion or motivation that the McNamara reference be combined with Slamon and Veltri to arrive at the present invention.

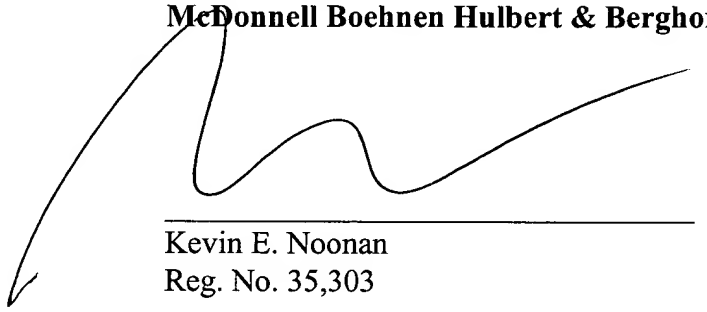
Applicant respectfully contends that rejection on 35 U.S.C. § 103 grounds has been traversed by their argument herein, and request that this rejection be withdrawn.

Conclusion

In view of the above remarks, the application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone call would expedite the prosecution of this application,

the Examiner is invited to call the undersigned attorney. In the case where the application is not put into condition for allowance after the Examiner considers the amendments and arguments made herein, Applicants respectfully request that the Examiner provide them the opportunity to have a formal interview, preferably un person, to discuss the matter further.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff



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